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Production and Characterization of a Monoclonal Antibody against Recombinant Glutathione S-Transferase (GST) of *Fasciola gigantica*

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SUMMARY **A monoclonal antibody (MoAb) against a recombinant glutathione S-transferase (rGST) of *F. gigantica* was produced in BALB/c mice. Reactivity and specificity of this monoclonal antibody was assessed by ELISA and immunoblotting. Six stable clones, namely 3A3, 3B2, 3C6, 4A6, 4B1 and 4D6 were obtained, All these MoAb reacted with rGST and native GST at a molecular weight of 28 kDa and found to be IgG_1, κ-light chain isotypes. These MoAb cross-reacted with *Schistosoma mansoni* and *Schistosoma japonicum* antigens at molecular weights of 28 and 26 kDa, respectively, but no cross-reactions were detected with antigens of *Eurytrema* and *Paramphistomum* spp. The localization of GST in metacercaria, 7-week-old juvenile and adult *F. gigantica* was performed by immunofluorescence technique, using MoAb as well as polyclonal antibody (PoAb) to the native protein as probes. In general, all clones of MoAb gave similar results and the pattern was quite similar to staining by PoAb. The fluorescence was intense, which implied the presence of a high concentration of GST in the parenchymal tissue in all stages of the parasite. However, the parenchymal cells were not evenly stained which implied the existence of subpopulations of this cell type with regard to GST production and storage. In addition, in adult and juvenile stages a moderate fluorescence was present in the basal layer of the tegument, while light fluorescence was observed in the caecal epithelium, cells in the ovary, testis and vitelline gland of the adult. In the metacercaria stage, in addition to parenchymal tissue, the tegument and tegumental cells were stained relatively more intense with MoAb and PoAb than in other stages.**

Glutathione-S-transferases (GSTs) are a family of multifunctional enzymes that occur in most forms of life and are considered to serve in the intracellular detoxification of oxygen free radicals, which may be generated by mutagens, carcinogens, other noxious chemical substances and even from ordinary metabolic reactions. GSTs are also proposed to play similar roles in helminthes, especially in protecting the parasites by inactivating the reactive oxygen free radicals and nitric oxide generated by the hosts' immune effector cells.[1,2,3] In mammals, a number of cytosolic GSTs have been shown to belong to four homologous multigene families designated ∝, μ, π and θ. The GST isoenzymes within each class have a high level of sequence conservation but can be distinguished by the difference in molecular weights, isoelectric points, immunological properties and specific substrate affinities.[4] In *F. hepatica*,[1,5] *S. mansoni*[6,7] and *S. japonicum*[8,9,10] GST isozymes in the ∝ class and the even more abundant μ classes have been characterized, and they show similarities in molecular masses and cross-reaction. In immunolocalization studies, GSTs were detected in all developmental stages of *S. mansoni*,[7] except in the intrauterine immature eggs. The antigen was reported to be present in the tegument, protonephridial cells, subtegumental parenchyma of male and

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female, and the dorsal tubercles of male tegument. Immature germinal cells in both sexes and the ootype in the female genital system were also found to contain GSTs. In adult *S. japonicum*[10] the enzyme was detected in parenchymal cells but not on the surface nor within the tegument matrix or caecal epithelium. In comparison, in *F. hepatica* GST isoenzymes were specifically confined to parenchyma, caecal epithelium[2] and the inner zone of the tegument syncytium,[5] while they were detected only in the parenchyma and excretory ducts of the newly excysted juvenile parasites.[2] A similar study has not yet been performed in *F. gigantica*. As a result of their highly vital role in protecting the parasites from attacks by hosts' immune effector cells, GSTs have been proposed as one of the ideal targets for immunological intervention in a variety of host-parasite systems, especially infections by *Schistosoma* and *Fasciola* spp.[11,12,13] The isolation and purification of GST from native source is a difficult and laborious process, hence recombinant protein has been synthesized by molecular cloning.[5,14] In the present study, a monoclonal antibody against recombinant *F. gigantica* GST was produced, characterized, used for probing the distribution of GST in this parasite's tissues at various stages of the life cycle. The cross reactivities with other trematode parasite antigens were tested in order to probe for their possible applications in immunodiagnosis and vaccine development.

MATERIALS AND METHODS

Parasite samples

Adult *F. gigantica* were removed from the bile ducts and gall bladders of condemned bovine livers at slaughterhouses. Other trematode parasites collected from the same group of cattle for cross reaction studies included *Paramphistomum* spp. from the rumen and *Eurytrema pancreaticum* from the pancreas. *S. mansoni* and *S. japonicum* were collected from mice infected with cercariae 8 weeks earlier. All parasite specimens were washed three times with Hank's balanced salt solution (HBS) containing 100 U/ml penicillin and 100 mg/ml streptomycin to remove all traces of blood, bile and contaminating microorganisms.

The metacercariae were obtained from infected *Lymnae ollula* snails. The snails were infected with miracidia and allowed to develop sporocysts and cercariae. The cercariae were shed from the snails and settled on 5" x 5" cellophane sheets, on which cercariae encysted themselves and developed into metacercariae. At day 45 metacercariae were examined under the stereomicroscope, brushed from the cellophane papers and washed several times with 0.85% normal saline and kept at 4°C until use.

Seven-week-old juveniles were obtained from infected male Swiss albino mice, each of which was orally infected with 20 metacercariae. The juveniles were collected from the liver in the seventh week after the infection, and washed several times with 0.85% normal saline before being used further.

Excretory-secretory antigen (ES) of adult *F. gigantica*

The ES-antigen was prepared by incubating freshly collected, living adult parasites in Hank's balanced salt solution (Gibco, USA) containing 10 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM EDTA at room temperature for 3 hours. The parasite eggs in the culture medium were removed by centrifugation at 5,000 x *g* for 20 minutes at 4°C. The supernatant was dialyzed in 0.01M PBS, pH 7.2 at 4°C for 24 hours using Spectra/Por 1 dialysis membrane (Thomas Scientific, USA) with molecular weight cut-off at 6 to 8 kDa. Then it was lyophilized, and kept at –20°C until use.

Tegumental antigen (TA) of adult *F. gigantica*

TA was obtained by extraction from live adult parasites with 1% Triton X-100 in Tris-HCl buffer, pH 8, for 30 minutes at room temperature. The extracting solution was collected and centrifuged at 5,000 x *g* for 20 minutes at 4°C to remove the parasite eggs which might be released during the extraction. The supernatant containing TA was collected and dialyzed in 0.01 M phosphate buffered saline (PBS), pH 7.2, at 4°C for 24 hours using Spectra/Por 1 membrane before it was lyophilized and kept at -20°C until use.

Whole body antigen (WB) of parasites

Whole adult parasites (*F. gigantica* and other trematodes) were homogenized in 0.01 M PBS, pH 7.2, which contained 0.5% Triton X-100, 10 mM PMSF and 10 mM EDTA, and extracted overnight with continuous rotation at 4°C. The suspensions were centrifuged at 5,000 x *g*, 4°C, for 20 minutes and the supernatants were collected, lyophilized, and stored at –20°C until use.

The protein contents of all fractions were determined by modified Lowry's method.[15]

Preparation of *F. gigantica* rGST

A 657 bp cDNA fragment encoding a GST of *F. gigantica* was cloned by RT-PCR.[14] It was reamplified by PCR to introduce a *Nde* I recognition sequence at the 5' end of the fragment. The reamplified GST DNA fragment was inserted in pBluescript SK(-) (Stratagene) and, after sequence verification, isolated

from the vector by digestion with the restriction endonucleases *Nde* I and *Bam*HI. Subsequently, it was subcloned into the *Nde* I and *Bam*HI sites of the expression vector pET-21a (Novagene). The subcloning procedure ensured that the expressed recombinant GST contained no additional amino acids as both start and stop codons were provided by the inserted DNA. Upon induction by IPTG (1 mM) rGST was detected by SDS-PAGE analysis in the soluble protein fraction. It was, thereafter, purified by affinity using the Redi-Pack GST purification Module (Pharmacia) following the protocol of the manufacturer.

Production of monoclonal antibodies (MoAb) against *F. gigantica* rGST

BALB/c mice were immunized subcutaneously with *F. gigantica* rGST in complete Freund's adjuvant at a dose of 25 μg in 100 μl per mouse. The second injection with a similar dose of the recombinant protein in incomplete Freund's adjuvant was given 3 weeks later. A final boost of 25 μg of protein in 100 μl PBS was given intravenously 2 weeks later. Hybridomas were produced by fusion of spleen cells from BALB/c mice immunized with *F. gigantica* rGST and mouse myeloma cells (P3/x63-Ag8). The hybridoma cells that grew successfully in the culture were monocloned by the limiting dilution method. Only the hybridoma clones that produced high titers of antibodies against rGST, as screened by indirect ELISA, were selected for immunoblotting. The antibody isotypes were determined by ELISA using the Mouse MonoAb-ID kit (ZYMED Laboratories, USA).

Immunoblotting

Immunoblotting was performed as described previously.[16] Briefly, rGST, TA, ES, and WB antigens were separated in 12.5% SDS-PAGE and blotted onto nitrocellulose membranes. As positive controls, the antigenic bands in each fraction were detected by immune sera of naturally infected cattle (CIS). Strips containing similar antigenic fractions were also screened by MoAbs and PoAb (rabbit anti *F. gigantica* GST- a gift from Dr. Terry Spithill, Monash University, Australia). For negative controls, culture fluid (CF) and normal mouse serum (NMS) were used. Antigenic molecules that reacted with CIS were detected by peroxidase-conjugated rabbit anti-bovine immunoglobulin, whereas the MoAb-antigen and PoAb-antigen complexes were detected by peroxidase-conjugated rabbit anti-mouse- and goat anti-rabbit IgG, respectively. The reaction was visualized by further incubation in 3,3' diaminobenzidine (DAB) and H_2O_2.

Immunolocalization of GST

Consecutive frozen sections of *F. gigantica* at each developmental stages (metacercaria, 7-week-old juvenile and adult) were cut in a cryostat and fixed with acetone at –10°C for 10 minutes. After washing with 10 mM PBS, pH 7.4, for 5 minutes, the sections were incubated with 0.1% glycine in 10 mM PBS, and subsequently with 4% BSA in 10 mM PBS to block nonspecific binding for 15 and 30 minutes, respectively. Thereafter, the sections were incubated with the MoAb or PoAb for 1 hour at room temperature and then rinsed 3 times with 10 mM PBS, pH 7.4, for 5 minutes each. They were finally incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse (for MoAb) or goat anti-rabbit (for PoAb) IgG for 30 minutes at room temperature. Finally, the treated sections were rinsed thoroughly with PBS, mounted in glycerol-PBS (9:1), and then observed under a Nikon HB 10101 AF fluorescence microscope.

RESULTS

Characterization of *F. gigantica* antigens by SDS-PAGE

F. gigantica proteins in the WB and the TA fractions were similar in appearance, and appeared to be highly complex and consisting of a vast number of bands ranging in molecular weights (MW) from below 14.5 kDa to more than 116 kDa (Fig. 1A). The major protein bands of the WB fraction which showed intense staining were at 66, 52, 37, 28 and 14.5 kDa while minor bands were found at 57, 45, 42 and 29 kDa. The tegumental antigens showed major bands at 57, 43, 41, 29, 28, 18 and 14.5 kDa. In contrast to the TA and WB, the ES fraction showed much fewer bands with the two most predominant ones at MW of 28 and 29 kDa; in addition a few light bands at high MW of 66, 64 and 58 and low MW of 14.5 kDa. The rGST appeared as a single band at 28 kDa (lane 4, Fig. 1A).

Monoclonal antibodies against rGST

Six stable clones of MoAb, designated 3A3, 3B2, 3C6, 4A6, 4B1, 4D6 were selected and expanded in culture flasks to obtain large volume of MoAb which were then gathered for further experiments. All MoAb exhibited specific binding to the rGST band of 28 kDa, although with varying intensities (Fig. 1B). The immunoglobulin classes and sub-isotypes of all MoAb selected were found to be kappa-IgG_1.

When tested by WB from the adult *F. gigantica*, all MoAb reacted with a single native band at 28 kDa, which also reacted with PoAb (Fig. 1C; 2A). When tested against WB, TA and ES fractions all MoAbs exhibited similar patterns as illustrated by the reaction of clone

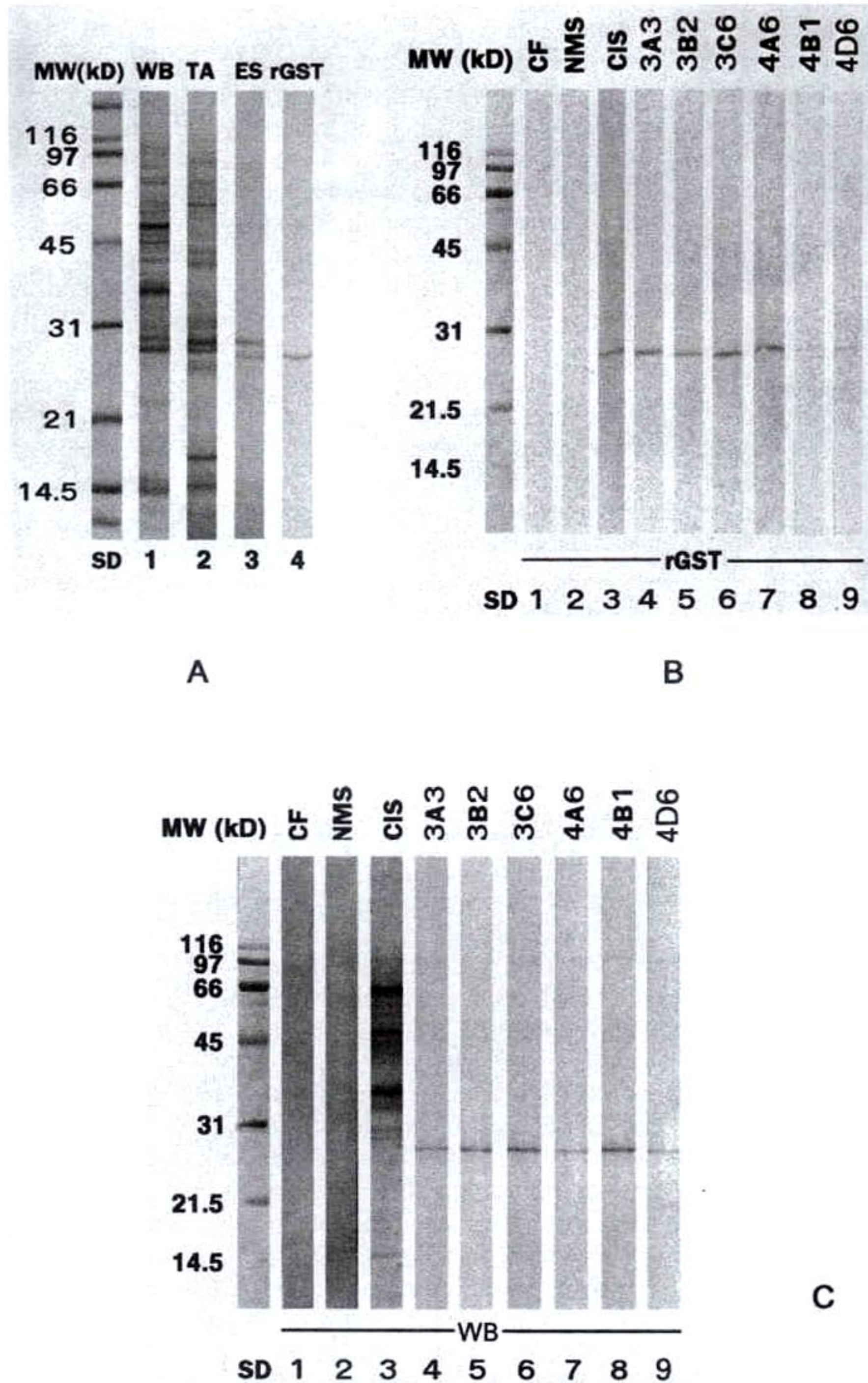


Fig. 1 (A) Coomassie blue stained 12.5% SDS-PAGE of *F. gigantica* antigens: lane 1-whole body fraction (WB); lane 2-tegument fraction (TA); lane 3-excretory-secretory fraction (ES); lane 4-recombinant GST (rGST). (B) Immunoblotting patterns of rGST reacted with myeloma culture fluid-CF (lane 1), normal mouse serum-NMS (lane 2), cattle infected serum-CIS (lane 3), MoAb 3A3 (lane 4), 3B2 (lane 5), 3C6 (lane 6), 4A6 (lane 7), 4B1 (lane 8) and 4D6 (lane 9). (C) Immunoblotting patterns of *F. gigantica* WB antigens reacted with myeloma CF (lane 1), NMS (lane 2), CIS (lane 3), MoAb 3A3 (lane 4), 3B2 (lane 5), 3C6 (lane 6), 4A6 (lane 7), 4B1 (lane 8) and 4D6 (lane 9). SD = standard molecular weights.

3A3, which reacted intensely with a single band in ES and WB at MW 28 kDa (Fig. 2A). In contrast, this band appeared very light in the TA fraction. When PoAb against native GST was tested against the WB, TA and ES fractions from adult *F. gigantica*, the PoAb also reacted with the native protein at 28 kDa which appeared as a doublet (Fig. 2A). In addition, there was another positive band at 24 kDa in WB against TA which was also detected by PoAb (lanes 8, 9, Fig. 2A). In the cross-reactivity study, the MoAb specifically reacted with a single protein band at MW 28 kDa of *S. mansoni* and 26 kDa of *S. japonicum*. In contrast, no positive band was detected when MoAb were reacted against the crude worm extracts from *Eurytrema* and *Paramphistomum* spp. (Fig. 2B).

Immunolocalization and distribution of GST

Since all MoAbs exhibited similar staining patterns, the data obtained from clone 3A3 were used as the representative of the group.

The most intense immunostaining by MoAb in the adult tissues was observed in the parenchyma (Fig. 3B, C). Moderate staining was observed in the middle and basal layer of the tegument, the tegument cell's cytoplasm and their processes, light staining appeared in the caecal epithelium, cells of the vitelline gland, ovary and testis, whereas the tegumental spines and muscle were not stained (Fig. 3B-E). The negative control experiment using myeloma culture fluid and normal mouse serum did not give positive staining, but yellowish autofluorescence appeared in the vitelline glands in a granulated pattern which may correspond to vitelline granules (Fig. 3A). The positive control experiment using PoAb showed very intense staining in the parenchyma tissue (Fig. 4B), moderate staining in cells of the vitelline gland (Fig. 4B), the tegument and the processes of tegumental cells running between negatively stained muscle cells (Fig. 4B, E). Cells of the testis, ovary and caecal epithelium exhibited moderate to weak staining (Fig. 4B-D).

Similar to adults, immunostaining of the juvenile worm with MoAb and PoAb was found to be intense in the parenchymal tissue, but moderate in the tegument, and light in the caecal epithelium (Fig. 3F; 4G). The negative control experiment using myeloma culture fluid did not exhibit any positive staining (Fig. 3A, 4F).

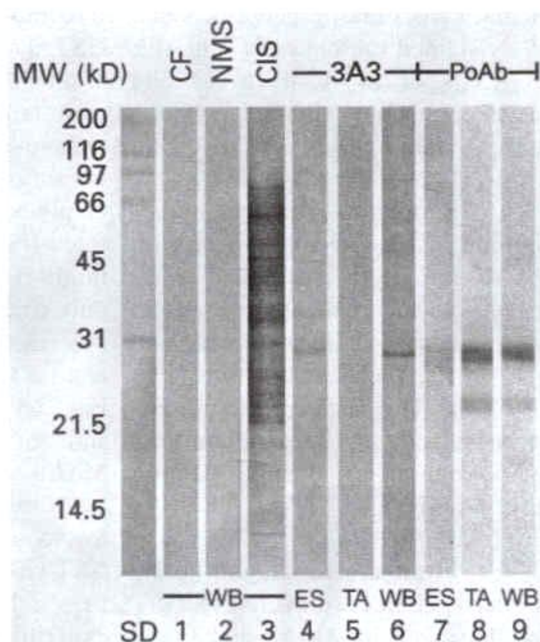
In metacercariae and newly excysted juvenile worms (NEJ), GST was localized by MoAb and PoAb in similar tissues. However, the tegument was relatively more intensely stained than in other stages and appeared as a narrow intense fluorescent layer around the parasites underneath the innermost layer of the cyst wall (Fig. 3H, 4I). Intense staining was clearly also observed in the cytoplasm of parenchymal and tegument cells, while their nuclei remained unstained (Fig. 3H, 4I). In contrast, caecal epithelium appeared to be lightly stained in comparison to that of adult and juvenile stages (Fig. 3H, 4I). Nonspecific weak autofluorescence appeared in the inner cyst wall, and stronger yellowish autofluorescence was also observed in the outer cyst wall, while the tissues in metacercariae (Fig. 3G) and NEJ (Fig. 4H) remained negative.

DISCUSSION

Characterization of monoclonal antibodies to rGST of *F. gigantica*

GST of adult *F. hepatica* have been purified and shown to consist of a mixture of at least three to five isoenzymes of MW 23-26.5 kDa, all of which showed N-terminal sequence variations.[1,5] This heterogeneity of *F. hepatica* GSTs was also reflected in the cloning and characterization of several cDNA encoding GST that showed a 59-89% identity at nucleotide levels and a 71-89% identity at the amino acid level.[17] In the present report, MoAb against *F. gigantica* rGST could detect only a single band of the SDS-PAGE separated-ES and WB antigens which might reflect the identification of only one isotype in these two antigen fractions, while PoAb could detect up to three isotypes (lanes 8, 9, Fig. 2A). In contrast to PoAb, the stain of the GST band detected by MoAb was very weak in the TA fraction, which might be due to the low concentration of this particular GST isotype in the tegument as confirmed by the immunofluorescence experiment. The presence of GST at a rather high concentration in ES material may be due to the need to detoxify oxidants generated from chemicals or host immune cells external to the parasites, thus this oxidant scavenging enzyme may be needed on the surface of the parasite though the secretory pathways are not yet known.[18] Tang *et al.*[19] reported that GST have been found in the ES materials of several genera of parasites, including *Dictyocaulus viviparus*, *Onchocerca volvulus* and *Brugia* spp., a lymphatic filarial nematode. Cervi *et al.*[20] identified glutathione S-transferase as an ES protein in *F. hepatica* adults, and it has been suggested that there is a low but continuous release of this enzyme into the ES.[21,22]

It has been established that cross-protection exists between *Fasciola* and *Schistosoma* species in many hosts. Hillyer *et al.*[23] and Christensen *et al.*[12] showed that *F. hepatica* infections in mice induced significant resistance to subsequent challenges with *S. mansoni*. Conversely, mice infected with *S. mansoni* showed significant resistance to the subsequent challenge with *F. hepatica.*[13] Furthermore, GSTs of *F. hepatica* (FhGSTs),



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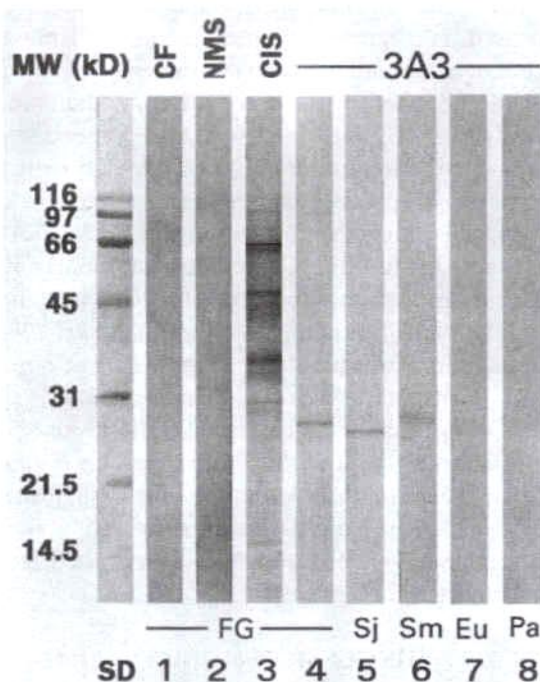

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Fig. 2 (A) Immunoblotting patterns of *F. gigantica* WB antigens reacted with myeloma CF (lane 1), NMS (lane 2), CIS (lane 3); ES, TA and WB probed with MoAb clone 3A3 (lanes 4, 5, 6) and PoAb (lanes 7, 8, 9). (B) Immunoblotting patterns of WB from *F. gigantica* (lane 4), *S. japonicum* (lane 5), *S. mansoni* (lane 6), *E. pancreaticum* (lane 7) and *Paramphistomum* spp. (lane 8) probed with MoAb 3A3. Lanes 1, 2 and 3 show WB patterns of SDS-PAGE separated-antigen of *F. gigantica* reacted with myeloma CF, NMS and CIS, respectively. Other clones of MoAb showed similar patterns (data not shown). SD = standard molecular weights.

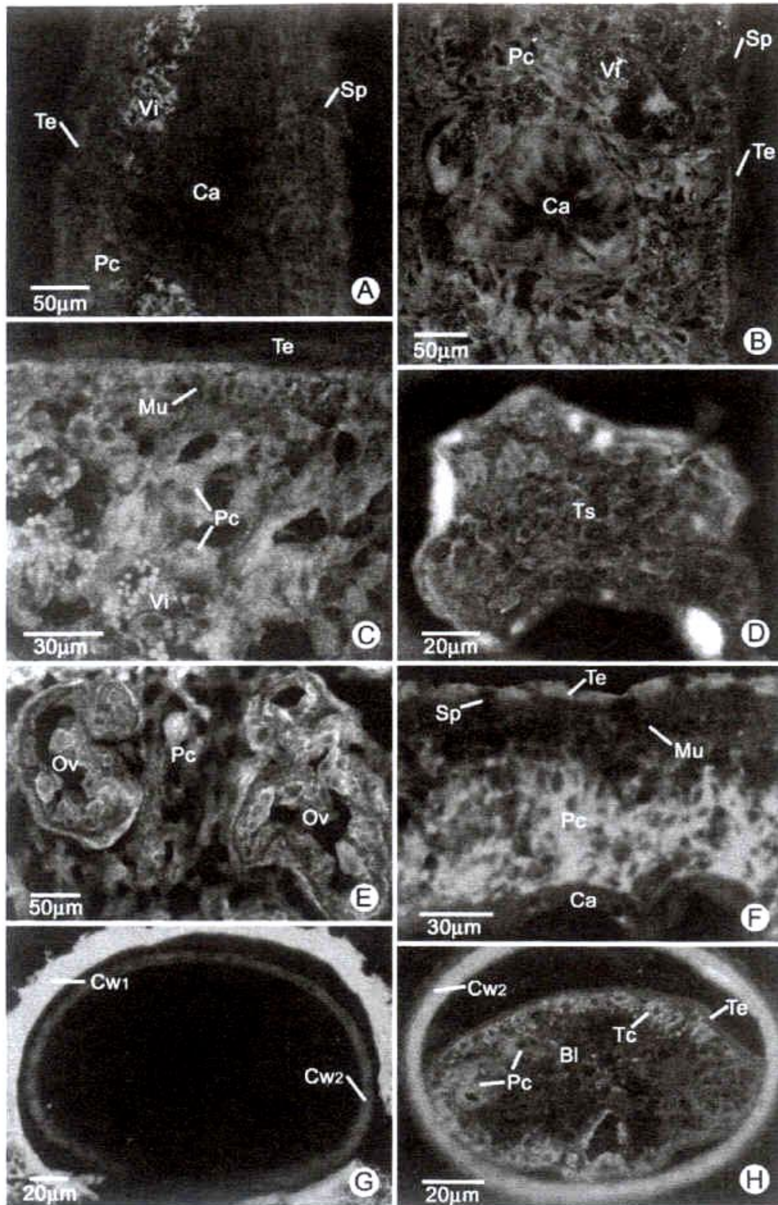

Fig. 3 Light micrographs of *F. gigantica* frozen sections stained by immunofluorescence technique, using MoAb as primary antibody and FITC-conjugated goat anti-mouse IgG as secondary antibody. (A) A control section of an adult parasite, using myeloma CF in place of primary antibody, showing granulated autofluorescence in vitelline cells (Vi). (B) Low magnification micrograph of an adult parasite, showing intense staining in parenchyma (Pc), moderate staining in tegument (Te), light staining in caecum (Ca) and vitellaria (Vi). (C) High magnification micrograph, showing intensely stained parenchymal cells (Pc), moderately stained tegument (Te), and lightly stained vitellaria (Vi). Note the intensely stained processes of parenchymal cells between unstained muscle cells (Mu). (D) A high magnification micrograph showing light staining of testicular cells (Ts). (E) A high magnification micrograph showing light staining of cells in the ovary (Ov). (F) Micrograph of a 7-week-old juvenile section, showing intense staining in parenchyma (Pc), moderate staining in tegument (Te), and light staining in the caecum (Ca). (G) Control section of metacercaria, using myeloma CF in place of primary antibody, showing yellow autofluorescence in the outer cyst wall (Cw1) and non-specific staining of the inner cyst wall (Cw2) while the metacercarial tissues are unstained. (H) Micrograph of a metacecarial section, showing intense staining in parenchyma (Pc) and tegumental cell (Tc). (Bl, bladder).

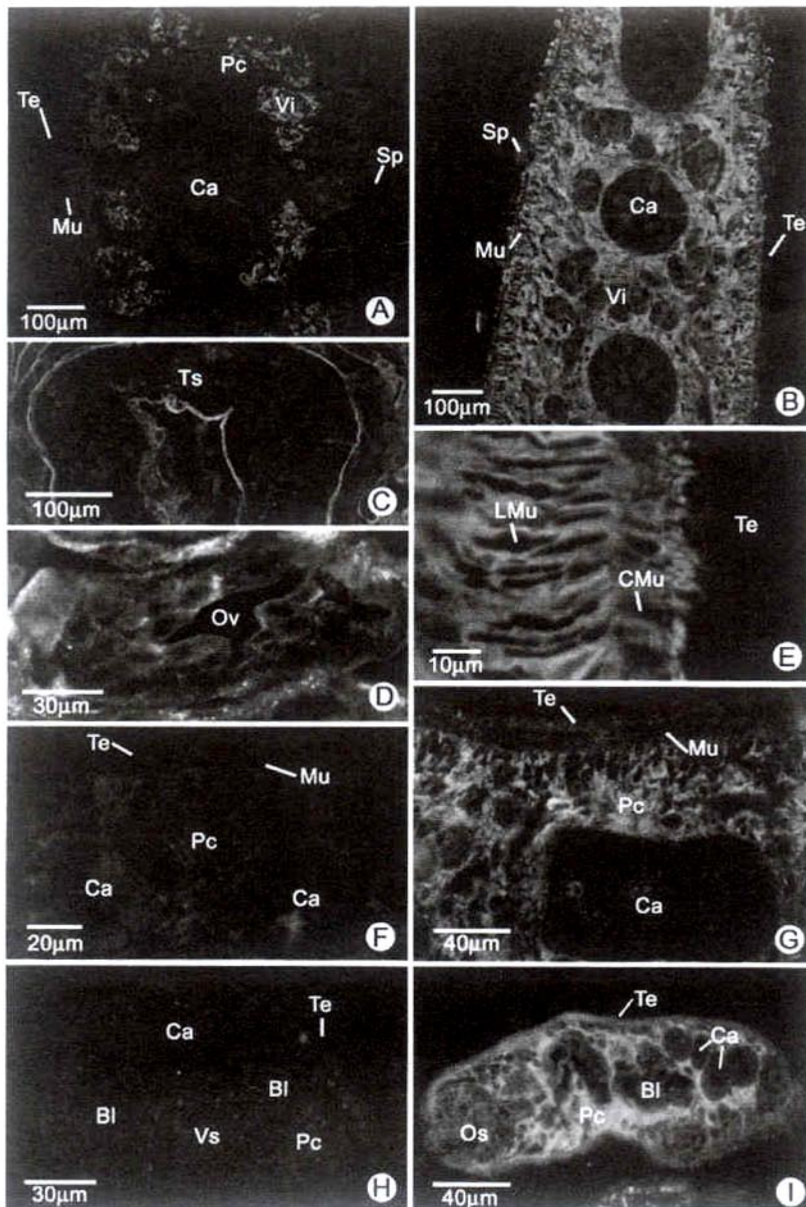

Fig. 4 Light micrographs of *F. gigantica* frozen sections stained by immunofluorescence technique, using PoAb (rabbit anti-native GST) as primary antibody and FITC-conjugated goat anti-rabbit IgG as secondary antibody. (A) Control section of an adult parasite, using myeloma CF in place of primary antibody, showing autofluorescence in vitelline cells (Vi). (B) Low magnification micrograph of an adult section, showing intense staining in parenchyma (Pc), and light staining in tegument (Te) caecum (Ca) and vitellaria (Vi). (C) A micrograph showing light staining of cells in the testis (T,s). (D) A high magnification micrograph showing light staining of cells in the ovary (Ov). (E) High magnification micrograph, showing intensely stained parenchymal cells and their processes running between muscle layers (LMu) towards the tegument (Te), and lightly stained tegument (Te). (F) Control section of a 7-week-old juvenile, showing no staining. (G) A micrograph of a 7-week-old juvenile, showing intense staining in parenchyma (Pc), light staining in tegument (Te) and caecum (Ca). (H) Control section of NEJ. (I) A section of metacercaria, showing intense staining in parenchyma (Pc), fairly intense staining in tegument (Te), and light staining in caecum (Ca). (CMu, circular muscle; LMu, longitudinal muscle; Os, oral sucker; Sp, spine; Vs, ventral sucker).

S. mansoni (Sm28) and *S. japonicam* (Sj26) were shown to have cross protection in experimentally vaccinated laboratory animals, including mice and hamsters.[24] It was also found that the putative gene encoding FhGSTs has 54 and 58% homology to Sj26 and Sm28 genes, respectively.[8,25] In the present study, the crude antigens of *F. gigantica*, *S. mansoni*, *S. japonicum*, *Eurytrema* spp. and *Paramphistomum* spp. separated by SDS-PAGE were immunoblotted with MoAbs against rGST, and the result showed that only a 28 kDa antigen of *S. mansoni* and a 26 kDa antigen of *S. japonicum* reacted specifically with these monoclonal antibodies. This indicated a high degree of cross-reactivity between *S. mansoni*, *S. japonicum* and *F. gigantica* GSTs, at least for this single isotype. In contrast, no cross reaction was found with antigens from even closely related trematode parasites, i.e. *Eurythema* and *Paramphistomum* spp. Hence, it is possible that these MoAb could be used for immunodiagnosis of fasciolosis and schistosomiasis which will be studied further.

Localization of GST

In the present study, GST expressed in various stages in the life cycle of *F. gigantica* were localized by indirect immunofluorescence using PoAb against native GST and MoAb against rGST as probes. In general, the results showed that the pattern of immunostaining by PoAb was very similar but more intense than staining by MoAb. From the fluorescence intensity it could be surmised that GST has the highest concentration in the parenchymal tissue in all stages of the parasite. Moreover, in the adult parasite GST was also detected at a moderate concentration in the middle and basal cytoplasm of the tegument. GST was present in low amount in cells of the vitelline gland, ovary, testis and the caecal epithelium. In juvenile parasites parenchymal tissue also showed a high concentration of GST, while the enzyme was present in a rather low concentration in the tegument and caecal epithelium. In contrast, GST was detected in a relatively high concentration in the tegument and tegumental cells of the metacercarial stage, in addition to parenchymal tissue. Since the MoAb that was produced in the present study could detect only one isotype the immunolabeling pattern may not be the complete picture of all native GSTs. However, its' staining is in general agreement with other reports on *F. hepatica*[2,5] which showed the existence of GST in the parenchymal cells and their cytoplasmic extensions in the subtegumental area, as well as in the caecum and excretory ducts of the adult and juvenile stages. However, there is still no report on GST expression and concentration in the metacercarial stage of *F. hepatica*.

For the *Schistosoma* spp., several studies have been undertaken to analyze the distribution of GST. Taylor *et al.*,[7] using immunoelectron microscopy, detected GST (Sm28) in the tegument, protonephridial cells and subtegumental cells of *S. mansoni*. Holy *et al.*,[26] using immunofluorescence technique, could detect SmGST only in the parenchyma, while Porchet *et al.*[27] could also detect the enzyme in the genital organs. Gobert *et al.*[10] performed the immunolocalization study of the 26 kDa and 28 kDa glutathione S-transferase within adult *S. japonicum*, and found that both proteins were localized within the parenchyma of both male and female parasites. However, there was no staining of this protein on the surface or within the tegument of either the male or female worms. The absence of GST in the caecal epithelium in *Schistosoma* spp. is in notable contrast to the positive finding of this enzyme in the caecum of *Fasciola* spp. as reported earlier[2,5] as well as in the present studies.

The presence of a moderate amount of GST in the tegument, in contrast to the lower amount in the caecal epithelium, may be due to the fact that this enzyme is needed for the parasite's protection from oxygen free radicals generated from the host immune cells, which are more likely to attack the tegument surface than the caecal epithelium. It is still not known how GST in the tegument is produced. Since tegumental cells provide the cytoplasmic mass for the tegument they may actually be the original source of GST, though the staining of the tegumental cells themselves was not intense except in the metacercarial and NEJ stages. Alternatively, GST may be provided by the parenchymal cells, which evidently synthesized and stored a large amount of GST. These cells branch profusely and always have the terminals of their slim processes in close contact with the tegument.[28] The passage of GST from parenchymal cell processes to the tegument is possible but there is still no proof for this cell to cell transfer. In addition, the abundance of GST in the parenchyma implicates that there is a very high degree of protection from oxidants' damaging effects within the parasite's body. This was supported in an experiment when the adult parasites were incubated with MoAb against rGST, and the tegument was severely damaged, but the adult parasites could still survive and regenerate the new tegument (unpublished observation). In the younger stages, especially NEJ there appeared to be a relatively much lower quantity of GST in the parenchyma than in adult as shown by immunofluorescence in this study, and by quantitation of specific activity of this enzyme (showing about x20 fold increase) in *F. hepatica*.[29] Hence a GST vaccine may have a higher chance of killing these young parasites. Because of its expression in all developmental stages of *F. gi-*

gantica and its moderate specificity to only *Fasciola* and *Schistosoma* species, rGST synthesized in this study could be considered as a candidate for both immunodiagnosis and vaccine development for these species, which will be studied further.

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